McGILL UNIVERSITY PUBLICATIONS

SERIES XIV (PHYSIOLOGY) No. 21

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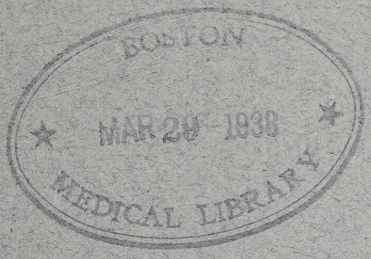

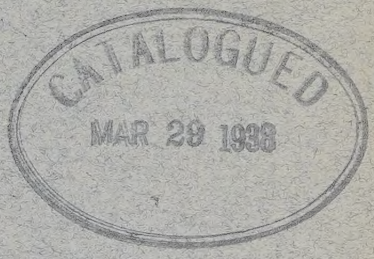

Reprinted from the American Journal of Physiology, Vol. 80, No. 2,
April, 1927, pp. 301-310.

MONTREAL, 1927

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 80, No. 2, April, 1927

THE EFFECTS OF LOWERED BODY TEMPERATURE AND OF INSULIN ON THE RESPIRATORY QUOTIENTS OF DOGS

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Received for publication October 30, 1926

Pembrey (1901) found that the respiratory quotient of the European marmot was often greater than unity during the period of feeding immediately preceding the onset of torpidity, and that the respiratory quotient fell to about 0.53 when the animal became dormant. He also pointed out that when the process of awakening began the respiratory quotient rose to about 0.75. Similar observations have been made by numerous other investigators. Marès (1892) recorded a respiratory quotient of 0.295 for an hibernating spermophile. In a previous communication the authors (1925) reported that a state simulating hibernation could be produced in dogs and cats by the combined effects of lowered body temperature and of insulin hypoglycemia. The experiments recorded here were carried out with the object of investigating the respiratory quotients and metabolic rates of dogs in this condition.

METHOD. The dogs were kept on a diet of beef heart for a week or two prior to the experiments. In every instance from 18 to 24 hours had elapsed between the last feeding and the time of use. Some of the animals were anesthetized with amytal, while the others received ether. The Folin-Wu blood sugar method was used. Deep rectal temperature was recorded, the bulb of the thermometer being inserted to a depth of about 8 cm. The body temperature of the animals was lowered by immersing them in a cold bath. Insulin was given subcutaneously in every case. Respiratory quotients and metabolic rates were determined by the Douglas bag method. When air was collected from animals that had not been anesthetized, a rubber mask fitting closely around the head was employed. Great care was taken to adjust the mask so that leakage was entirely prevented. When amytal or ether had been given, a tracheotomy was performed and a tracheal cannula was inserted. The mask or tracheal cannula was attached to a Y-piece which, in turn, was connected to the respiration valves by two 2-foot lengths of rubber tubing. The valves

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were placed in such a position that gravity tended to keep them closed. Since the flaps consisted of very thin aluminum discs, very little pressure indeed was required to effect their opening; while the possibility of their failing to close completely was practically eliminated. Further, the rubber tubes connecting the Y-piece to the valves served as traps, thus preventing loss of expired air should either of the valves leak. The expiration valve was attached to the Douglas bag in the usual manner. The bag was washed out every day or two with 1 per cent sulfuric acid solution, in order to reduce diffusion of carbon dioxide through the rubber to a minimum. After collecting the air the Douglas bag was emptied through a gas meter, a sample being collected at the same time. Pearce sampling tubes were employed. The expired air was analysed by means of the Haldane-Henderson gas analysis apparatus, duplicate determinations being made in nearly every case. The time of collection of expired air varied from 6 minutes to 1 hour, the longer periods being used where feasible.

The effect of amytal anesthesia on respiratory quotient and metabolic rate. Preliminary experiments were carried out to determine the effect of amytal anesthesia upon the respiratory quotient and metabolic rate of dogs. A sample of expired air was collected from an animal in the post-absorptive state, the dog having previously been trained to lie quietly during the collection. From 0.5 to 0.7 cc. of 10 per cent sodium amytal solution per kilogram of body weight was then injected intraperitoneally. Ten to fifteen minutes later, when surgical anesthesia had been induced, a second sample of expired air was collected. About an hour later a third determination of the respiratory quotient was made. The results of these experiments are shown in table 1. It will be noted that immediately after the administration of amytal the respiratory quotient fell to a marked extent, but that it returned to within $\pm$ 0.04 of its original value in about 30 minutes. Subsequent determinations during the next few hours showed that it remained practically constant once it had returned to its original level. Theodore Kruse (1923) found that ether narcosis produced similar changes in the respiratory quotient. We are unable at the present time to give an explanation of this effect of amytal. Since the percentage of carbon dioxide present in the expired air is decreased as a result of amytal administration, it seems probable that the low respiratory quotient observed immediately after the induction of anesthesia is not due to depression of the respiratory center and consequent storage of carbon dioxide in the blood. In order to ascertain whether amytal prevents the rise of respiratory quotient following glucose administration, an anesthetized animal was given several subcutaneous injections of glucose solution. The respiratory quotient of this dog was 0.65 35 minutes after amytal had been injected; whereas 4 hours later it was 0.88, two injections of 2 grams of glucose per kilogram having been given in the interim.

Amytal greatly reduces the metabolic rate. In the case of dog 52 the metabolic rate before amytal was given was 2.578 calories per kilogram per hour, and 106 minutes after the administration of this drug, it was 0.695 calorie per kilogram and per hour.

The effect of lowered body temperature on respiratory quotient and metabolic rate. In order to determine the effects of lowered body temperature upon the respiratory quotient of dogs, experiments of the following type

TABLE 1

	R.Q. BEFORE AMYTAL	AFTER AMYTAL		AFTER AMYTAL	
		Time	R.Q.	Time	R.Q.
		minutes		*minutes*	
Dog 52	0.82	16	0.65	106	0.86
Dog 53	0.67	12	0.42	35	0.65
Dog 54	0.75	8	0.48	27	0.73
Dog 61	0.61	14	0.48	156	0.65
Dog 63	0.77			60	0.74

TABLE 2

	R.Q. AT NORMAL BODY TEMPERATURE	AFTER COOLING		REMARKS
		Temperature	R.Q.	
		°C.		
Dog 41		25.3	0.53	Amytal anesthesia. No shivering
Dog 43	0.73	28.0	0.68	Amytal anesthesia. No shivering
Dog 44	0.70	25.0	0.32	Amytal anesthesia. No shivering
Dog 46	0.71	25.7	0.40	Amytal anesthesia. Slight shivering
Dog 46	0.71	24.5	0.45	Amytal anesthesia. No shivering
Dog 46	0.71	25.3	0.57	Amytal anesthesia. No shivering
Dog 46	0.71	29.5	0.61	Amytal anesthesia. No shivering
Dog 60	0.73	22.0	0.33	Amytal anesthesia. No shivering
Dog 62	0.68	20.6	0.58	Ether anesthesia. Slight shivering
Dog 62	0.68	20.0	0.53	Ether anesthesia. No shivering
Dog 68	0.77	26.5	0.34	Amytal anesthesia. No shivering
Dog 71	0.76	22.5	0.42	Ether anesthesia. No shivering
Dog 72	0.69	24.5	0.49	Amytal anesthesia. Slight shivering

were carried out. An animal was given amytal and about an hour later a sample of expired air was collected. The dog was then immersed in a bath of ice water. Cooling was continued until the animal ceased to shiver; for, as will be pointed out later, the respiratory quotient of a dog increases to a marked extent whenever shivering occurs. In an amytalized dog the shivering reflex disappears when the body temperature has fallen to a level varying from 22°C. to 30°C., depending upon the depth of anesthe-

sia and upon the condition of the dog. As shown in table 2, the respiratory quotient falls far below 0.70 when the body temperature of a dog is lowered to a sufficient extent to abolish shivering. In the case of dog 44 it fell to 0.32, and in that of dog 60 to 0.33. Similar experiments were carried out under ether anesthesia. Here the respiratory quotient at normal body temperature was determined before the animal was anesthetized. Etherization was continued until the dog's temperature had fallen to about 25°C. Expired air was collected an hour or two after ether had been discontinued. As in the previous experiments, the respiratory quotient was greatly lowered (table 2). In order to be certain that these low quotients were not caused by storage of carbon dioxide in the blood, three samples of air were collected in succession from the same dog. The animal remained attached to the respiration valves throughout the experiment. The carbon dioxide to oxygen ratio was the same (within 0.01) in the three samples.

TABLE 3

	TEMPERATURE	CO_2 EXCRETED PER KILOGRAM PER HOUR
	°C.	cc.
Dog 47	38.0	302.6
Dog 58	35.0	172.0
Dog 43	28.0	150.7
Dog 41	25.3	62.0
Dog 44	25.0	41.1
Dog 46	29.5	119.5
Dog 46	25.7	15.9
Dog 46	25.3	16.5
Dog 46	24.5	19.0

Further, the percentage of carbon dioxide present in the expired air was less after the animal had been cooled than it was when the dog's temperature was normal. This indicates that in a dog cooled to the temperatures mentioned, the respiratory function was adequate to prevent the carbon dioxide content of the blood from being increased; in point of fact it would seem that the carbon dioxide content was somewhat decreased. Further evidence bearing on this point will be brought forward later.

Two dogs were anesthetized with amytal and given glucose subcutaneously. These animals were then cooled by exposure to cold air. In spite of the fact that these animals had received carbohydrate, the respiratory quotients decreased. The quotient of dog 58, 1 hour and 40 minutes after amytal had been injected, was 0.65. Two hours and a half later, after the animal had received 30 grams of glucose its respiratory quotient was 0.60 and its temperature had fallen to 35°C.

According to Pembrey (1901) the only way in which respiratory quo-

tients of less than 0.70 can be accounted for is to assume that fat is being converted into carbohydrate. It would seem, therefore, that lowering the body temperature of an animal causes a change in the type of its metabolism.

As shown in table 3, lowering the body temperature of a dog decreases the metabolic rate. The animals mentioned in this table were not shivering at the time when their metabolic rates were determined, and all of them were anesthetized with amytal. Metabolic rates have been expressed in terms of cubic centimeters of carbon dioxide excreted per kilogram and per hour; since there are no data on record concerning the calorific value of a liter of oxygen when the respiratory quotient is below 0.70. It will be seen that the metabolic rate does not decrease in the manner that would be expected taking van't Hoff's law into consideration.

TABLE 4

	ANIMAL SHIVERING		ANIMAL NOT SHIVERING		REMARKS
	Temperature	R.Q.	Temperature	R.Q.	
	°C.		°C.		
Dog 43	30.0	0.73	28.0	0.68	Amytal anesthesia
Dog 44	27.8	0.62	25.0	0.32	Amytal anesthesia
Dog 47	36.0	0.79	38.0	0.67	Amytal anesthesia
Dog 47	28.7	0.64	28.5	0.54	Amytal anesthesia
Dog 60	25.0	0.58	22.0	0.33	Amytal anesthesia
Dog 62	20.4	0.64	20.0	0.53	Ether anesthesia
Dog 71	22.0	0.69	22.5	0.42	Ether anesthesia. Shivering abolished by curare
Dog 49	38.5	0.73	38.8	0.57	Amytal anesthesia
Dog 42	28.0	1.02	38.7	0.72	Amytal anesthesia

The effect of shivering on respiratory quotient and metabolic rate. When an amytalized dog is immersed in a cold bath, it begins to shiver almost immediately, and continues to do so until its temperature falls to a level varying between 22°C. and 30°C. However, if ether has been used during cooling, and sufficient time allowed for the effects of the anesthetic to pass off, shivering does not disappear until the temperature of the animal has fallen to about 21°C. The respiratory quotient of several dogs was determined just before and just after shivering had been abolished by cold. The results of these experiments are shown in table 4. It will be noted that the quotient was considerably higher in every case when the animal was shivering. The respiratory quotient of dog 47 was determined about 1 hour after amytal administration. Its temperature at this time was 38°C., and its respiratory quotient 0.67. This animal was then immersed

in cold water. It began to shiver almost at once, and the respiratory quotient rose to 0.79, in spite of the fact that its temperature had fallen to 36°C. Richet (1893) has reported similar findings concerning the effect of shivering on the respiratory quotient.

Some of the results quoted above are open to the criticism that it was necessary to lower the body temperature of the dogs a degree or two further in order to abolish shivering. Hence the decrease in respiratory quotient attributed to the abolition of shivering may have been due in part to the fact that the temperature of the animal was lower after shivering had disappeared. In a subsequent experiment it was decided to keep the temperature of the animal constant and to abolish shivering by means of curare. Accordingly, a dog was etherized and cooled in the usual manner. When its body temperature had fallen to 22°C., the animal was still shivering vigorously. Its respiratory quotient was 0.69. Three mgm. of curare per kilogram of body weight were given intravenously. Shivering and respiration stopped almost immediately. Artificial respiration by means of the

TABLE 5

Dog 60. Amytal anesthesia

TEMPERATURE	R.Q.	REMARKS
°C.		
38.5	0.73	Shivering
30.2	0.73	Shivering vigorously
27.7	0.68	Shivering vigorously
25.0	0.58	Shivering vigorously
22.0	0.33	No shivering

Brodie pump was commenced at once. The pump ran very slowly and was adjusted so that the amount of inflation produced by each stroke was small. Twenty minutes after curare had been injected, a sample of air was collected. Although the temperature of the animal had risen 0.5°C., the respiratory quotient had fallen to 0.42. Artificial respiration was continued for 45 minutes longer, at the end of which time another sample of of air was collected. Analysis of this sample showed that the respiratory quotient was 0.44; the temperature of the dog at this time was 23.4°C. Since this determination agreed very closely with that made 45 minutes earlier, it was apparent that mere storage of carbon dioxide in the blood was not responsible for the low ratio of carbon dioxide to oxygen observed. This in conjunction with the preceding experiments proves beyond question that the occurrence of shivering is associated with a rise of respiratory quotient.

The rise of respiratory quotient coincident with the occurrence of shivering indicates, in our opinion, that shivering is associated with the metabo-

lization of carbohydrate. Our reason for saying carbohydrate, rather than protein or fat, is that the respiratory quotient of a dog often rises to unity when shivering is very vigorous. Thus, in the case of dog 42 (table 4) the carbon dioxide to oxygen ratio was 0.72 one hour and a half after the animal had received amytal. When the temperature of the dog had been lowered to 28°C., shivering was very vigorous and the respiratory quotient rose to 1.02.

It will be seen from table 4 that the respiratory quotients of some of the cooled dogs were below 0.70 even when they were shivering vigorously. This probably indicates that fat is being converted into carbohydrate and that carbohydrate is being utilized at the same time. Further evidence in support of this contention is shown in table 5. The respiratory quotient of dog 60 fell progressively from 0.73 to 0.58 as the body temperature was lowered, yet this animal shivered vigorously from the time of immersion until its temperature had fallen to 22°C. When this animal ceased to

TABLE 6

Dog 47. Amytal anesthesia

TEMPERATURE	CO_2 EXCRETED PER KILOGRAM PER HOUR	REMARKS
°C.	cc.	
38.0	302.0	No shivering
36.0	1726.0	Shivering vigorously
34.3	1166.0	Shivering vigorously
28.7	472.2	Shivering vigorously
28.5	141.1	Shivering slightly
23.8	22.3	No shivering

shiver, the utilization of carbohydrate decreased, as shown by the fact that the respiratory quotient fell from 0.58 to 0.33.

Richet (1893) and subsequent investigators have shown that the occurrence of shivering is associated with a great increase in the metabolic rate. Similar results were obtained in the course of our experiments (table 6). In the case of dog 47 the increase amounted to about 500 per cent.

The effect of insulin on respiratory quotient and metabolic rate. The administration of insulin to a normal dog causes the respiratory quotient to rise, in some cases to greater than unity (Dickson, Eadie, Macleod and Pember, 1924). The question whether insulin would have a similar effect on a cooled animal presented itself. With this in view dog 41 was given amytal and its temperature lowered to about 25°C. The protocol of this experiment follows:

Dog 41. Weight, 9.6 kgm.

10:35 Given 0.45 cc. of 10 per cent sodium amytal solution per kilogram
10:55 Immersed in cold bath. Shivering vigorously
12:50 Body temperature 26.3°C. Removed from bath. No shivering
1:18 Body temperature 24.5°C. Blood sugar 108 mgm. per cent
2:51 Body temperature 25.3°C. No shivering. Blood sugar 134 mgm. per cent
3:10 R.Q. 0.53. M.R. 62 cc. CO_2 per kilogram per hour
4:15 Given 4 units of insulin per kilogram. Body temperature 24.5°C. Placed on warming table
5:55 Body temperature 25.4°C. No shivering
6:45 Body temperature 27°C. Removed from warming table
8:17 Body temperature 27°C. Blood sugar 64 mgm. per cent. No shivering
8:20 R.Q. 0.60. M.R. 90.2 cc. CO_2 per kilogram per hour
10:20 Blood sugar less than 20 mgm. per cent
11:10 R.Q. 0.64. M.R. 88.1 cc. CO_2 per kilogram per hour. Body temperature 26°C. No shivering
1:18 R.Q. 0.66. M.R. 85.1 cc. CO_2 per kilogram per hour. Body temperature 26°C. No shivering

This experiment demonstrates that in a dog whose body temperature has been lowered, the administration of insulin is followed by a rise of respiratory quotient. It is to be noted that this animal did not shiver after it had been removed from the bath.

The authors (1925) have shown that the injection of insulin into a dog abolishes the capacity for shivering. As indicated above, the cessation of shivering is associated with a decrease in respiratory quotient. It was, therefore, of interest to determine the respiratory quotients of dogs in which shivering had been abolished by means of insulin hypoglycemia. Such experiments were carried out on two dogs. The results in the case of dog 73 are shown in table 7. When the animal was shivering vigorously the respiratory quotient was 0.65 and the metabolic rate was 425.6 cc. of carbon dioxide per kilogram per hour. One hour and thirty-four minutes after insulin had been injected, shivering ceased and the respiratory quotient fell to 0.43 and the metabolic rate to 86.6 cc. of carbon dioxide. The reappearance of shivering after glucose had been administered caused a marked rise both in the respiratory quotient and in the metabolic rate. From the above experiment it is apparent that the respiratory quotient of a dog falls when shivering is abolished by means of insulin, just as it does when this reflex is abolished by cold, anesthesia or curare. As would be expected, the abolition of shivering causes the metabolic rate to be greatly reduced.

Summary. Lowering the body temperature of a dog is in itself sufficient to cause the respiratory quotient to fall to a level as low as 0.32, provided that the metabolization of carbohydrates associated with shivering is suppressed. The occurrence of shivering is coincident with a marked rise in the respiratory quotient, and when this form of muscular activity is abol-

ished—whether by lowered body temperature, anesthesia, curare, or insulin —there is a corresponding fall in the respiratory quotient. It has also been shown that the metabolic rate of a dog that has been given insulin after its body temperature has been lowered to 25°C. or less is reduced to from 15 per cent to 25 per cent of the basal rate at normal body temperature. It is evident that the respiratory quotient produced in a dog by the combined action of lowered body temperature and of insulin hypoglycemia has about the same magnitude as that of the hibernating marmot. Further, the respiratory quotient of the cooled hypoglycemic dog rises to about the same extent when glucose is injected as it does during the awakening process of the marmot.

TABLE 7

Dog 73

TIME	TEMPERATURE	R.Q.	CO_2 EXCRETED PER KILOGRAM PER HOUR	REMARKS
	°C.		cc.	
10:04				Given 0.5 cc. amytal per kilogram
11:03	38.5	0.55	161.1	
11:39				Immersed in cold bath
12:50	26.3			Removed from bath
2:36	26.4	0.65	425.6	Shivering vigorously. Blood sugar 139 mgm. per cent
3:10	31.0			Given 10 units insulin per kilogram
4:04	26.0	0.43	86.6	Shivering ceased. Blood sugar 65 mgm. per cent
5:40	26.0			Given 2 grams glucose per kilogram
5:50	26.5	0.73	435.6	Shivering vigorously

CONCLUSIONS

1. There is a fall in the respiratory quotient of a dog immediately after the administration of amytal. The respiratory quotient returns to its original value in from 30 to 60 minutes. The metabolic rate is greatly reduced by amytal anesthesia.

2. Lowering the body temperature of a dog is in itself sufficient to cause the respiratory quotient to fall to a level as low as 0.32, provided that shivering has been suppressed. Even while the animal is shivering, the respiratory quotient may fall to a level far below 0.70. Therefore, lowering the body temperature of an animal causes a change in the character of its metabolism.

3. The occurrence of shivering is associated with a rise in the respiratory quotient indicating increased utilization of carbohydrate.

4. The abolition of shivering, whether by means of insulin, curare,

lowered body temperature, or anesthesia, causes the respiratory quotient of a dog to fall.

5. The administration of insulin to a cooled dog that is not shivering causes the respiratory quotient to rise.

The expenses of this research were defrayed in part by a grant from the James Cooper Fund of McGill University for Research in Experimental Medicine.

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